

TRANSFORMERS

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*Future of
Medicine*

SPECIAL REPORT

By reprogramming DNA inside harmful microbes, biologists are turning them into patient-saving drugs

By Michael Waldholz

Journalist **Michael Waldholz** led a team of reporters who were awarded a Pulitzer Prize in 1997 for their coverage of AIDS. He lives in New York State's Hudson Valley.



IN A FEW MONTHS A SMALL GROUP OF VOLUNTEERS will gulp down billions of tiny, toxin-gobbling contraptions to cure a crippling disease. The devices are not made from the usual machine parts of metal, wire or plastic. They are rebuilt organisms: bacteria, reconstructed from the inside out to perform an intricate feat of medical care.

together like transistors in an electronic gadget. Wedged into the ordinary *E. coli*'s genome, the circuit replaces the bacterium's normally slow ammonia-consuming mechanism with a super-charged version, an ammonia-gobbling

beast that switches on when it senses the low oxygen levels characteristic of the human gut.

If Synlogic's genetically altered bugs can gorge on ammonia in humans as they have in tests with mice, then tossing down the bacterial concoction every day for the rest of their lives may enable UCD sufferers to survive practically symptom-free. The amplified bacteria will cure a devastating genetic disease, arising in more than 100 new patients a year in the U.S., for which there is not now an adequate remedy. "We've replaced a missing physiological function with a whole new kind of therapy," says Paul Miller, Synlogic's chief scientific officer. "It's an amazingly powerful way to attack disease." Miller's company is crafting similar circuits against more common illnesses such as irritable bowel disease, inflammatory and immune disorders, and even cancer.

Transformed bacteria have a key advantage over more typical drugs, which are chemical-based pills where the only thing doctors can change is the dose. The bacterial circuits can be easily fine-tuned to increase potency or to extend or reduce the time of activity, and they can be turned down so that they become safer. Bacteria's natural ability to sense and respond to their environment also makes them target-specific: they can be programmed to release a therapeutic substance only when at the site of disease. This selective action may avoid the side effects typical of pills that act throughout the body.

Researchers working at Synlogic, a Cambridge, Mass., biotechnology start-up, will hand patients daily doses of a pill or a drink loaded with billions of *Escherichia coli* bacteria. These kinds of microbes typically bustle about our innards, occasionally causing infections but generally living innocuous lives. What makes these particular *E. coli* different is that scientists have revamped chunks of their DNA—genetic instructions that tell the microbes what to do—to transform these tiny cells into engines relentlessly driven to devour poisonous loads of ammonia in patients' bodies.

The patients have urea cycle disorder (UCD), a liver enzyme deficiency that can kill newborns and make adults sick. They are born with a faulty gene that produces defective enzymes unable to break down the nitrogen in high-protein foods such as meat, eggs and cheese. Normal enzymes turn excess nitrogen into a chemical called urea, which gets peed away. But for those with the genetic disorder, the excess nitrogen does not leave the body. Instead it generates toxic levels of ammonia that accumulate in circulating blood and inflict havoc when they hit the brain.

Synlogic's engineered bacteria will guzzle extra ammonia. Gut bacteria take in small amounts of ammonia already, using its nitrogen for growth. The retrofit from scientists gives the microbes a new genetic "circuit," a series of genes and regulatory bits of DNA such as volume controls and on/off toggles wired

IN BRIEF

By taking control of a microbe's genetic circuitry, biologists can turn it into a medical treatment that switches on and off in particular situations.

The change involves connecting protein-coding genes and switches, in the way electrical circuits link conductors, resistors and capacitors.

Bacteria are now being modified with new circuits that let them treat genetic diseases, attack tumors and detect antibiotics.

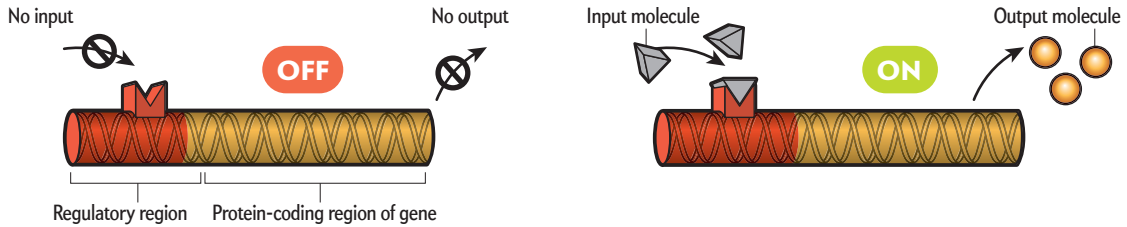
Living Circuits

Genes—and other segments of DNA that switch genes on and off—can be wired together in novel ways. They work like electrical circuits that run household gadgets. These DNA circuits, though,

can be placed within living organisms like bacteria to control the microbes' behavior. With that control, synthetic biologists can turn the organisms into living medicine.

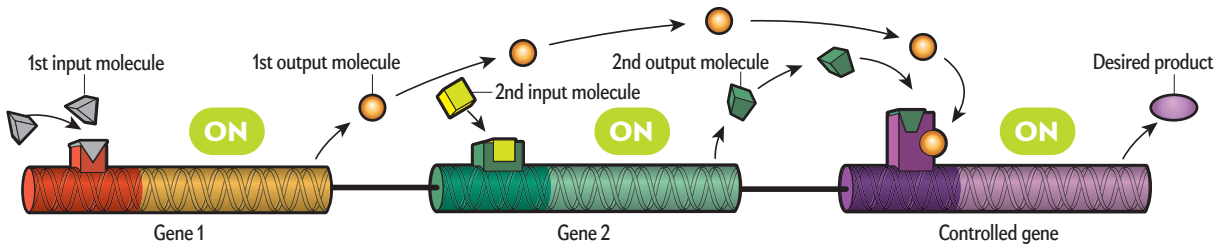
A Simple Switch

In a basic circuit, a gene can be turned on by a particular signal to produce a useful substance. The gene (orange) is linked to a regulatory region (red). When that region has no input, the circuit is off and produces nothing. But if that region is stimulated by an input molecule (gray), it turns the gene on and makes a desired output molecule.



Adding Complex Logic

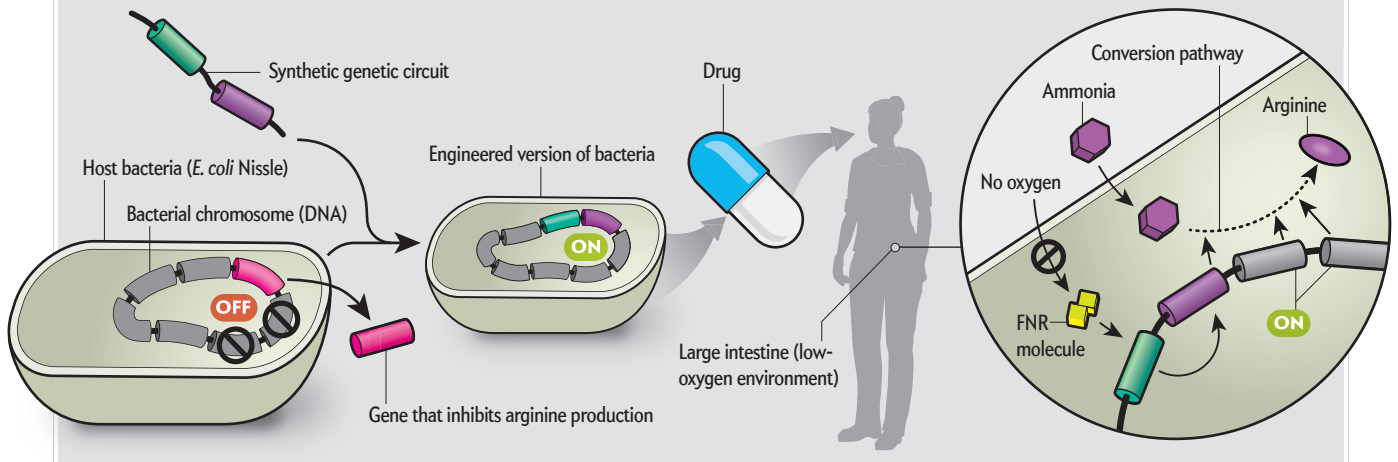
The switch can be combined with other elements to give biologists more advanced control—logic—over what a microbe does. One example is this “AND” circuit. Gene 1 (orange), when switched on, sends output to the controlled gene (purple). Gene 2 (green), also switched on by an input molecule, sends output to the controlled gene as well. The controlled gene switches on only when stimulated by both gene 1 and gene 2.



Building Bacteria to Fight an Enzyme Disorder

Patients with urea cycle disorder have an enzyme deficiency that lets toxic levels of ammonia build up. Biologists are treating this by making *Escherichia coli* bacteria eat the ammonia. The microbes are engineered to produce large amounts of an amino acid, arginine, and they need to consume ammonia to make it. First a gene (pink) that inhibits extra arginine production is turned off. Another gene (green) is added, and it

switches on when stimulated by a protein called FNR (yellow). FNR only does this in a low-oxygen environment such as the human intestines. When the entire synthetic circuit is placed in bacteria, they become arginine-producing machines only when stimulated by ammonia and low oxygen levels. The dual control ensures bacteria do this inside the body, not after they are excreted into the oxygen-rich outside world.



The bacteria also may be able to replenish themselves within the human body, something no pill can do. They must still pass safety tests, and researchers acknowledge that they must show that their genetically enhanced bugs will not get unleashed dangerously into the environment. The Food and Drug Administration has given Synlogic the go-ahead to try the therapy in people this year because the strain of *E. coli* being used in the UCD therapy has long been safely prescribed as an oral probiotic to treat inflammatory bowel disease. If the human tests pan out, the company's therapy-in-a-germ will represent the first clinical application to emerge from a relatively new branch of genetic engineering called synthetic biology.

The field rides on advances in manipulating DNA, giving scientists new laboratory tools to link stretches of DNA together and produce effects more powerful than simply changing one gene. "Synthetic biology is now producing some impressive accomplishments," says James Collins, a professor of medical engineering at the Massachusetts Institute of Technology and a leading researcher in the field. Human cells, for instance, have been fitted with enhanced DNA circuits to pump insulin into the bloodstream more precisely than daily injections for diabetics. *Salmonella*—the bacterium associated with food-poisoning outbreaks—has been rejiggered to sneak up on cancer cells and unload a cargo of toxic drugs. The DNA-circuit approach can also diagnose disease: researchers in Boston recently redesigned a microbe to alert doctors to early sepsis infections brewing in the blood of hospitalized patients. Existing tests rarely pick up the problem until patients are much sicker and hard to treat.

The new technology has the potential to be transformative not just for bacteria but for medicine itself. "Biomedicine sits on the cusp of a new revolution in medical care," says Wendell Lim, director of the Center for Systems and Synthetic Biology at the University of California, San Francisco. "Microbial and human cells are becoming versatile therapeutic engines." It was not always such a rosy picture, however.

ENGINEERING BIOLOGY

FOR THE PAST 40 YEARS SCIENTISTS have used genetic engineering to discover and manipulate genes to reveal the intricate machinery that rules all life. But they were limited in their understanding of how the different parts fit together and worked in real life. Things that looked good in a test tube fell apart when tried in a real cell or animal. When synthetic biology got started, there was a lot of early hype, Collins admits. But beginning

Unnatural Responsibilities

Synthetic biology offers unusual rewards and risks

By Kevin M. Esvelt

The bold dream of synthetic biology is a world in which all living things can be reliably engineered in ways that help everyone and everything. In this dream, we can use genetics to program living organisms: "if condition A is met, then do action B." To give a near-term example, bacteria might produce a medicinal protein only in the presence of indicators of a particular disease.

Why use living systems and not a vat of chemicals? Because natural systems routinely perform complex chemistry that scientists can only envy, and they do it at room or body temperature, without the need for toxic chemicals or outside aid. Better still, living factories are far more energy-efficient than anything made of silicon and metal. Biology is fast, clean and green. And we should use such systems because people and ecosystems are alive, and the best way to repair life is with life. To fight an evolving pathogen, use an evolving cure.

There are problems, though, in bending nature to our own ends. Adopting an organism to work for us means it is using energy that could otherwise be spent replicating, so it will not reproduce as well as competitors. Evolution will constantly select for faster-reproducing mutants that no longer do what we want. Biology's greatest strength is its capacity to replicate and evolve, but that also presents the greatest challenge.

One way around this is to incorporate limits on the ability to change,

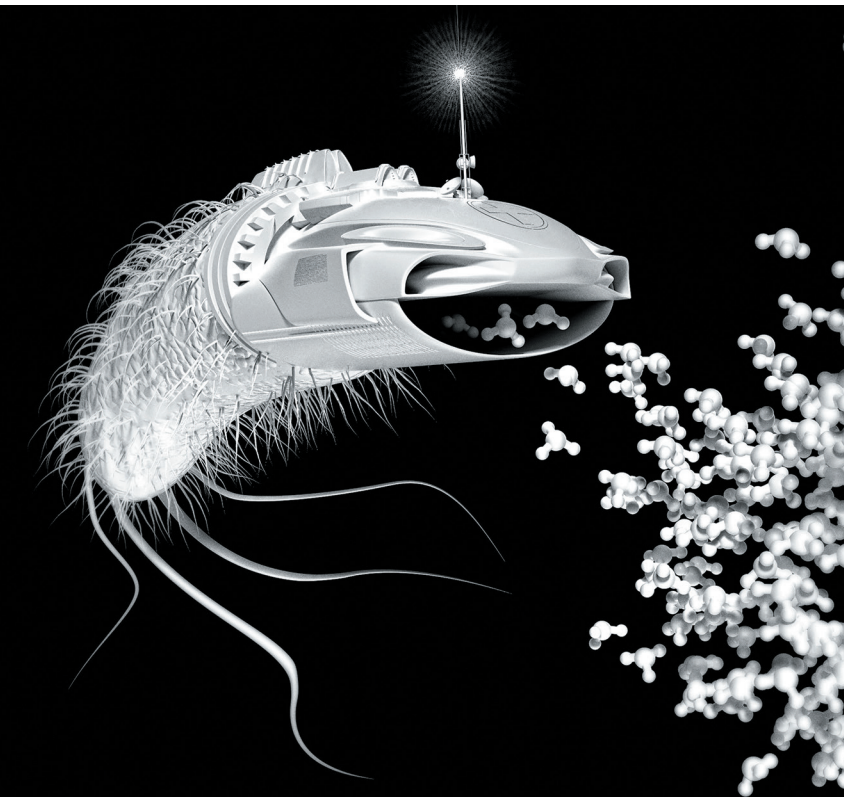
particularly for those few cases where our changes might be able to spread in the wild. For example, one approach is to employ unnatural amino acid tethers: they make essential proteins within cells wholly dependent on chemicals that do not exist in nature. If the amino acids are withheld, the proteins will not function, and the bacteria cannot grow out of control. We are also better at building within the scope of evolutionary limits: microbes are now programmed to release a burst of complex molecules and then die, mostly avoiding evolutionary selection against production. Cellular pathways can be reworked to eliminate most unwanted side effects. Engineered viruses that target bacteria will kill invading pathogens, multiply until the invaders are gone and then stop, leaving the patient untouched.

We must also be careful to make sure benefits always outweigh the risks of reworking organisms. Mistakes are inevitable. Thus, the projects have to be worth it, especially the earliest examples that must justify the technology to the world. Bacteria can be built to make a slightly cheaper flavor of vanilla, but is that a significant boon to humanity or the environment? This is likely not enough to be a pioneering example of a novel technology or to justify its use. On the other hand, building cells that can selectively destroy cancer or cure diabetes is something everyone can get behind.

The greatest biological risk to civilization stems from pandemics of infectious disease. Until now, these were inevitable, but we might soon use biotechnology to stop them. Ordinarily, a person's body confronts an invading pandemic pathogen by evolving its own defenses, creating a whole series of antibodies in the hope that one will effectively neutralize the invader. It is a

about 17 years ago, he and like-minded biologists, fueled by advances in sequencing and synthesizing DNA, have been employing the newly discovered genes and other DNA elements as interchangeable components to design and build medical applications that actually work outside a glass dish.

Part of the change has come from scientists with a bent for tinkering like engineers. "There's been a convergence of new



process of trial and error that takes time; this is why you are typically sick for three to four days before getting well. Sometimes that is just too long, and people die. A better strategy is to give the human body a head start: Take the genes for several known protective antibodies, put them into the harmless shell of a virus and inject that virus into people. The virus enters their cells, which then start to churn out already optimized protective antibodies against the invader, ending the threat.

Finally, as scientists we need to respect the fact that engineering life

unsettles many people. That means we must consider social risks as well as technical ones. We cannot just explain what we are doing—that only convinces other scientists. Instead we must relate why we care, who could benefit and what the risks may be. Above all else, we should actively invite concerns and criticism from the earliest stages because no matter how great our expertise, we cannot reliably anticipate every consequence on our own. At its best, science is a fundamentally shared undertaking. If we are to engineer life, let us all decide how to do it together.

Biologist Kevin M. Esvelt leads the *Sculpting Evolution* research group at the Massachusetts Institute of Technology's Media Lab.

ideas in the past few years that's driving the field," says Jeff Hasty, who co-directs the BioCircuits Institute at the University of California, San Diego. Hasty started his science career 20 years ago with a Ph.D. in physics. Only partly joking, he describes himself now as a "hybrid computational/molecular biologist." Synthetic biology is populated with folks such as Hasty, who embrace an engineer's inclination to "make stuff," he says.

"Much as an electrical engineer uses conductors, resistors and capacitors to create new electrical devices," Collins says, "we put together the components of biology—genes, proteins, RNA, transcription factors and other DNA—to create a particular function."

Collins notes that electronic gadgets are useful models for understanding genetic circuits. Consider an air conditioner thermostat. It senses an input—warming air temperature—and responds with an output—turning on the AC. When the air is cooled, the thermostat switches the machine off. Single-cell microorganisms such as bacteria survive in a similar way. Ever alert to an input, say, the presence of a competing germ, a bacterium responds with an output, secreting a natural antibiotic to kill its enemy.

The circuit builders of synthetic biology separated from straight genetic engineers as a result of coincidental insights by Collins and another research team. In 2000 Collins's lab, which was then at Boston University, reported making a genetic "toggle switch," one of two synthetic gene networks published in *Nature* in January of that year. The twin reports (the other was from a group at Princeton University) are generally cited as launching synthetic biology because they showed that "we could take parts of cells and link them together to generate a novel circuit the way an engineer might," Collins says. (It is no coincidence that, at the time, he was surrounded by circuits. He was running a bioengineering lab that was designing mechanical limbs for the disabled. Today Collins works at synthetic biology facilities in three different institutions in the Cambridge area. And he has trained about two dozen scientists—among them Hasty—who now have their own operations.)

In the years following the first primitive DNA-based switches, the still small community of synthetic biologists entered into a can-you-top-this competition, cooking up increasingly complex circuits that harnessed cells' natural sense-and-respond behavior. "As we went along, we learned, more than we first realized, how remarkably versatile the cell is," U.C.S.F.'s Lim says. He describes the cells as adaptable automotive "chassis" into which researchers can swap different genetic engines to carry out therapeutic functions.

One of the first commercial applications emerged in 2006 from scientists led by Jay Keasling of the University of California, Berkeley. Backed with a \$42.6-million grant from the Bill & Melinda Gates Foundation, Keasling's lab refashioned the metabolic pathways of ordinary baker's yeast with lab-designed circuitry that turned sugar molecules into a critical ingredient for making the malaria drug artemisinin. Previously, the precursor

molecule for manufacturing the drug was extracted by hand from sweet wormwood plants native to Asia, a costly process that made the drug too expensive for use in poor regions where malaria is rampant. “It was a breakthrough,” Collins says. “It was the first time a network of genetic material, not just one gene at a time, was used to transform a microbe—the yeast—into a solution for an important real-world problem.”

BROKEN CIRCUITS

BUT IT DID NOT LAUNCH a revolution. At about that time, J. Craig Venter, a famous genome scientist and co-founder of Synthetic Genomics in La Jolla, Calif., joined the synthetic biology fray, giving the technology its first public star. His highly publicized objective, which garnered a whopping \$300-million investment from Exxon in 2009, was to make gasoline from algae found in pond scum. In 2010 Keasling received a \$134-million grant from the Department of Energy to fund research aimed at coaxing yeast cells to synthesize diesel from chemicals in sugar plants. Earlier that decade Keasling had co-founded Amyris, a biotech company in Emeryville, Calif., to commercialize the alternative fuel technology.

Both projects wound up giving synthetic biology a bad rap. After four years, Exxon and Venter, as well as Amyris, essentially gave up the synthetic oil project. The cost of scaling up commercial production, as compared with the current low price of oil and natural gas, has forced Amyris and several other biofuel-from-microbes start-ups to put the venture on hold. These companies were disasters for investors. Amyris and different synthetic biotech companies launched between 2005 and 2010 on the promise of making oil from bugs continue to produce notable advances in biocircuitry design. But their new genetic circuits are not as widely celebrated. Instead these one-time rock stars of synthetic biology are reconstructing microbes to fabricate chemicals used in manufacturing solvents and lubricants, as well as the principal ingredients for cosmetics, fragrances, detergents and over-the-counter health products.

While Wall Street investors and the science media largely focused on the headline-grabbing dreams—and the less dreamy waking reality—of biofuels from bugs, without fanfare Collins and his colleagues spent much of the new century’s first decade working out technical hurdles for what was to come next: better medicine. After years of tedious experiments, in 2010 Collins engineered a bacterium that, in lab tests, weakened drug-resistant germs enough to make them vulnerable to existing antibiotics.

At about the same time, Tim Lu, another Collins postdoctoral protégé (with a Ph.D. in electrical engineering and computer science from M.I.T., as well as a medical degree from Harvard University), embedded circuits into a different kind of microbe, a virus that infects bacteria. Certain hard-to-treat infections arise when bacterial colonies encircle themselves within a gooey, protective biofilm. These bacteria may have evolved the biofilm to fend off the marauding viruses, which are called bacteriophages. Lu designed his circuit in the virus with a gene that codes for a

biofilm-degrading enzyme. Lu’s circuit also programmed the bacterial viruses to sense the presence of biofilm, infiltrate its defenses and respond by off-loading the film-busting enzyme.

Lu and Collins realized it might take years to perfect their infection fighters. But they also thought their bacteria might be ready sooner for another commercial use. In a meeting in 2013, Lu and Collins told a gathering of biotech funders at Atlas Venture in Cambridge that their labs’ genetically enhanced microbes could be transformed into living sentinels able to provide early detection of disease within the human body or contaminants in the air or water.

The Atlas executives, however, were taken by another, related

The circuit is designed to first fabricate a cancer drug inside the bacterium. It then directs the microbe to slip into the interior of a tumor, carried by the bloodstream, and self-destruct. When the microbe bursts apart, it releases its payload of drugs.

idea. They envisioned greater profits if the bacteria could be wired not simply to act as sentinels but to sense a health problem within the human gut—and then generate a therapy to treat it. The idea for Synlogic was born. In early 2015, about six months after the company hired its first researchers, it used Collins and Lu’s inventions to create an early version of the UCD therapy.

“I’ve been in the pharmaceutical industry a long time, and I’ve never seen a pharmacologic go from a scientist’s idea to clinical testing in so short a time,” says Bharatt Chowrira, a consultant to Synlogic.

REPURPOSED PARTS

THE TREATMENT’S COMPONENT is an especially clever circuit assembled with genetic parts that biologists uncovered during decades of *E. coli* research. The Synlogic circuit changes the bacterium’s usual ammonia-to-nitrogen-to-cell growth mechanism into a factory to churn out an amino acid called arginine. The researchers chose arginine because its cellular manufacturing demands more nitrogen than other amino acids. The need to make arginine turned the bacterium into an ammonia-gulping organism because it was desperate to take in nitrogen. With the circuit embedded into its genome, the microbe winds up producing “5,000 times more arginine than the normal

strain of bacteria,” says Jose-Carlos Gutiérrez-Ramos, Synlogic’s chief executive.

The circuit depends on a switch, a sequence of DNA that responds to a protein called FNR. Like the thermostat in an air conditioner, FNR is sensitive to changes in the bacteria’s surroundings. It enables *E. coli* to respond to an environment that lacks oxygen. When FNR senses that the bacteria are in a low-oxygen environment such as the large intestine, it turns on genes the microbes need to thrive. When the bacteria move outside the body, where there is plenty of oxygen, FNR is silent. This is a safety mechanism designed to prevent runaway organisms with high growth rates. Once the microbes exit the intestines and hit our oxygen-rich atmosphere as feces, the entire system shuts down, and the *E. coli* die.

There was one problem, though, Synlogic’s Miller says. *E. coli*’s genome contains a “repressor switch,” a gene called *argR*, that shuts down arginine production when it senses the bacteria have enough. So designers needed a mechanism to deactivate *argR* in their new circuit. Synlogic researchers accomplished this by knocking out the long DNA sequence that surrounds and includes *argR* with a nearly identical stretch of DNA in which the *argR* gene is deleted.

Several synthetic biologists have come up with other genetic circuitry to deliver anticancer drugs deep inside tumors. U.C.S.D.’s Hasty has armed a strain of *Salmonella* bacteria that is not harmful to humans with a special set of genetic instructions. Hasty’s experimental cancer therapy takes advantage of recent research that found that some bacteria often reside inside tumors. Scientists believe but are not certain that bacteria that naturally circulate in the bloodstream are attracted to tumors “because the environment provides a safe refuge from the immune system,” Hasty says.

Hasty’s genetic program forces the *Salmonella* to carry out a two-step process. The circuit is designed to first fabricate a cancer drug inside the bacterium. It then directs the microbe to slip into the interior of a tumor, carried there by blood the tumor needs for nourishment. At a moment directed by the circuit, the *Salmonella* self-destructs. When the microbe bursts apart, it releases a payload of drugs. “Sort of like a kamikaze mission,” Hasty says.

In another ingenious bit of designing, Hasty added several genetic components to make the therapy self-renewing. “We introduced into the bacteria a ‘quorum-sensing’ system that can detect when *Salmonella* reproducing inside the tumor achieve a certain population,” he says. When the multiplying microbes reach a high-enough density, the quorum sensor triggers the release of a protein that slices the *Salmonella* apart from the inside, spilling out the anticancer drug. This act of suicide kills most but not all of the *Salmonella*. Those that remain begin multiplying again, driving the cycle to repeat itself over and over.

The idea of attacking a tumor cell from within is especially attractive because most chemotherapy drugs work by eating away at a cancer cell’s outer walls. In a study in mice, the bacterial therapy did not work any better than standard chemotherapy when delivered alone, Hasty says. “But when we combined it with chemotherapy, we observed decreases in tumor sizes and a 50 percent increase in life expectancy in mice with a type of metastatic cancer,” he notes.

SEARCHING FOR APPROVAL

THE *SALMONELLA* WORK is still being refined. Synlogic’s UCD treatment is much further along, and the FDA’s approval process for this first therapy to involve genetically modified microbes is being scrutinized closely. The agency has released rules to regulate the microbe-based therapies under a new category it calls “live biotherapeutic products.” Unlike other medicines (with the exception of some vaccines), the new therapies are composed of organisms that are alive and have the potential to mutate as they reproduce. Because of this, the FDA wants assurance that the makeup of the therapies will not vary from one batch to another. In addition, it wants proof that the microorganisms cannot survive in the environment by themselves, as Synlogic claims. “Many of us are watching how Synlogic is handled by the regulators,” Hasty says. “If they can’t get their therapy approved, we may all be in trouble.”

An FDA review process for cells refashioned to detect disease, not produce new compounds within the human body, most likely will be faster and less expensive than one for a medical treatment. Many emerging synthetic biology projects are aimed at repurposing bacteria to diagnose the earliest presence of an ailment. “Gut bacteria can be engineered to sense, remember and report on their experiences as they pass through the intestine,” says Pamela Silver, a founding member of Harvard’s department of systems biology. Silver’s lab has created a proof-of-principle diagnostic tool composed of a genetic circuit that enables bacteria to identify the presence of an antibiotic in the digestive system of mice. The circuit produces a fluorescent signal that is visible in fecal waste if the antibiotic is active.

“This synthetic circuit demonstrates our ability to build a living diagnostic—in this case, exposure to antibiotic,” Silver says. The eventual goal is to use the technology to detect potential disease activity within the intestines. “The human intestine is a ‘dark’ place—difficult to explore yet the site of much activity affecting daily health and debilitating diseases, inflammation being one of most prevalent,” she says. Current diagnostics for digestive ills are invasive and costly.

A living diagnostic, Silver says, offers a cheap, potentially more sensitive approach. And if it passes muster, new functions can be added. “We also believe the diagnostic circuits can be further engineered to deliver treatment for bowel disease at the site of inflammation,” she says. “The power of the new circuits is creating all manner of possibilities.” ■

MORE TO EXPLORE

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